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MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: August 1, 1997

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SUBJECT: Antimicrobial Activity of Lovastatin and Related Drugs

This report is in response to your request, made during the lovastatin meeting held on June 9, 1997, for an evaluation of the published literature relevant to possible antimicrobial activity of lovastatin and related drugs. I have reviewed the literature relevant to the possible antimicrobial activity associated with the anti-hypercholesterolemia drug, lovastatin. In addition, I have reviewed and included literature reports on antimicrobial activity of related members in the "statin" class of drug products. The database searched for these literature reports was Medline for the years 1962 through June 12, 1997. Other databases have not been searched for information.

BACKGROUND:

In the preparation of this report, I have focused on the legal basis for the classification of a drug as an antibiotic drug as defined in Section 507 (a) of the Federal Food Drug, and Cosmetic Act. This legal description defines an antibiotic drug as "... any drug intended for use by man containing any quantity of any chemical substance which is produced by a microorganism and has the capacity to inhibit or destroy microorganisms in dilute solution (including the chemically synthesized equivalent of any such substance)." Therefore, to be determined an antibiotic drug, a human drug must possess the following properties:

1) It must be a drug intended for use by man which is produced by a microorganism or it may be any chemically synthesized equivalent of any such substance.
2) It must have the capacity to inhibit or destroy microorganisms.
3) It must demonstrate the capacity to inhibit or destroy microorganisms in dilute solution.

These characteristics of antibiotic drugs have been carefully considered for the purpose of determining if the reported antimicrobial activity of lovastatin and related drug products is sufficient to warrant their reclassification as antibiotic drug products

There are many analogues of "statin" drugs and related chemical substances that exhibit anti-cholesterolemia activity reported in the literature. However, this review will only address antimicrobial activity relevant to "statin" class drug products indicated for anti-hypercholesteremia activity submitted to FDA for marketing approval determinations. Currently, there are six drug products of the "statin" class, indicated for the treatment of hypercholesterolemia, under review or previously approved for marketing by the Center for Drug Evaluation and Research (CDER). Of the six, five, including Mevacor (lovastatin, MK-803, mevinolin, monacolin K), Zocor (MK-733, simvastatin, synvinolin), Pravachol (CS-514, SQ 3100, pravastatin, eptastatin), Lescol (fluvastatin sodium), and Liptor (atorvastatin calcium), have been approved for marketing (Package Inserts Merck, August 31, 1987; Merck, December 23, 1991; Bristol Myers Squibb, October 31, 1991; Sandoz, December 31, 1993; Park Davis, December 17, 1996, respectively). Baycol (cerivastatin sodium tablets) is currently under review (Bayer, NDA 20-740).

Fluvastatin, atorvastatin and cerivastatin are all manufactured by synthetic processes (Package Inserts and NDA 20-740) and as such do not fit the definition component requiring antibiotic drugs to be produced by microorganisms. Therefore, these drug products cannot be classified as antibiotic drugs and, consequently, will not be evaluated for antimicrobial activity in this review. Lovastatin, simvastatin and pravastatin are all drug substances which are either produced by microorganisms or are chemically synthesized equivalents of such substances (Germershaven, et al., 1989; Tobert, 1987; Tsujita, et al., 1986; Sitori, 1990; Alberts, 1988; Alberts, et al., 1980; Alberts, 1990). In addition, all have the capacity to inhibit or destroy microorganisms (*vide infra*). Therefore, all three of these drugs fit the first two definitions required for classification as antibiotic drugs However, the third requirement for antibiotic classification requires that these drugs must demonstrate the capacity to inhibit or destroy microorganisms in dilute solution. Interpretation of this requirement is somewhat problematic in that the term dilute solution and the kinds of microorganisms to be inhibited have not been defined. However, there appears to be a consensus within the agency and by some of the regulated drug industry that the microorganisms inhibited should be organisms that are causative agents of human clinical infections. In addition, the term dilute solution has been generally accepted as the drug concentration in preclinical studies that elicits inhibitory activity against microorganisms that correlates with clinically relevant human tissue drug concentrations. Human tissue drug concentrations considered relevant are those that are achieved from doses administered to the human target populations for the indicated use of the drug. The data from published literature relevant to interpretation of drug concentrations that "... inhibits in dilute solution ..." are summarized and evaluated in this report.

Data on "statin" antimicrobial activity from human studies have not been reported in the literature. Therefore, for this reason, this review contains only antimicrobial activity data generated from *in vitro* cell culture and *in vivo* animal model studies. During the review of these literature reports, it became clear that the preclinical antimicrobial activity data alone were insufficient to permit a rational interpretation of possible antibiotic activity associated with lovastatin and related drug products. For example, information on the experimental design of the studies, assays used for determination of activity, and studies on the mechanism of drug action were found to be important parameters when attempting to extrapolate *in vitro* activity results to expected clinical circumstances. In addition, species variability with respect to drug pharmacokinetics, pharmacodynamics, metabolism, elimination, bioavailability, tissue distribution and drug interaction potential were found to be relevant to the interpretations of antibiotic activity potential with respect to the definition "... inhibits in dilute solution ...". Therefore, to the extent possible and within the time-frame available, an effort has been made to provide this information in instances where it was deemed to be of value for the interpretation of parameters relevant to "statin" class drug products' potential antimicrobial activity expression

HISTORY:

In 1971, the Japanese researchers, Akira Endo and Masao Kuroda, began a search for inhibitors of microbial origin that would inhibit the rate limiting enzyme, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CoA reductase), in the biosynthetic pathway for cholesterol (Endo, et al., 1976a; Endo, 1985a; Endo, et al , 1985b; Endo, 1992). They anticipated that certain microorganisms would produce inhibitory products that would interfere with synthesis of required sterols or other isoprenoids required for growth of other microorganisms They hoped that these products would be effective in inhibiting *de novo* cholesterol biosynthesis and have the potential for reducing plasma cholesterol levels in

hypercholesterolemic humans. By 1973, several compounds that were effective in inhibiting HMG-CoA reductase, including ML-236A, ML-236B (compactin, mevastatin), and ML-236C, had been isolated from cultures of *Penicillium citrinum*. In 1976, after documentation that these inhibitors reduced cholesterol *in vitro* (Endo, et al., 1976a; Kaneko, et al., 1978, Alberts, 1988) and *in vivo* in animal models (Endo, et al., 1976a; Endo, et al., 1992) the first human subjects were treated (reviewed by Endo, 1992; Endo, et al., 1976b; Endo, et al., 1988; Tsujita, et al., 1986). Promising results in lowering plasma cholesterol in these early human studies led to human clinical trials ultimately resulting in the March, 1987, U.S. Food and Drug Administration approval of Mevacor (lovastatin) for the treatment of hypercholesterolemia (Approved Drug Products with Therapeutic Equivalence Evaluations. 14th Ed. 1994. US Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Drug Evaluation and Research).

The first publication suggesting that antimicrobial activity was associated with inhibitors of HMG-CoA reductase isolated from fungi appeared in 1976 (Brown, et al., 1976). The authors, citing a reference that was "in preparation", reported that compactin (mevastatin), a potent HMG-CoA reductase inhibitor, was isolated from a culture believed to be *Penicillium brevicompactum* and was detected by its antifungal activity. However, antimicrobial data for the drug (compactin), utilized for the investigations conducted by Brown et al., were not presented in that publication. An intensive computer search of the Medline database for the publication cited "in preparation" was unsuccessful. Evidently, it was never published; thus, a determination regarding the authenticity of the report cited by Brown cannot be made.

The first report of antimicrobial activity attributable to lovastatin was published in August, 1988 (Ikeura, et al., 1988) Thus, although the rationale for the search for these compounds was based upon an antibiotic principle (substance produced by a microorganism that inhibits other microorganisms), at the time of lovastatin's approval by FDA in March, 1987, reports including data on antimicrobial activity of "statin" drugs were not available in the published literature. Consequently, lovastatin was approved as a non-antibiotic drug under Section 505 of the Federal Food, Drug, and Cosmetic Act for its anti-hypercholesterolemia activity. Simvastatin was subsequently approved for its anti-hypercholesterolemia activity in December, 1991. Only three publication were found in the literature on simvastatin antimicrobial activity (Grellier, et al., 1994; Coppens et al., 1995a; Coppens et al., 1995b). Pravastatin was approved for its anti-hypercholesterolemia activity in October, 1991. Antimicrobial activity associated with pravastatin was not found in the literature searches conducted. However, because of structural and mechanism of action similarities to lovastatin and simvastatin, it is predictable that similar levels of antimicrobial activity, as has been reported for the other "statins", may exist for pravastatin.

The question under consideration in this report is the following: now that antimicrobial activity for lovastatin and simvastatin has been reported in the literature, are the published data sufficient to meet the antibiotic drug definition of "... inhibits in dilute solution ..." and, if so, should lovastatin and related drugs be considered for reclassification as antibiotic drug products under Section 507 of the Federal Food, Drug, and Cosmetic Act? The following data evaluation is intended to provide a reference framework for making that determination.

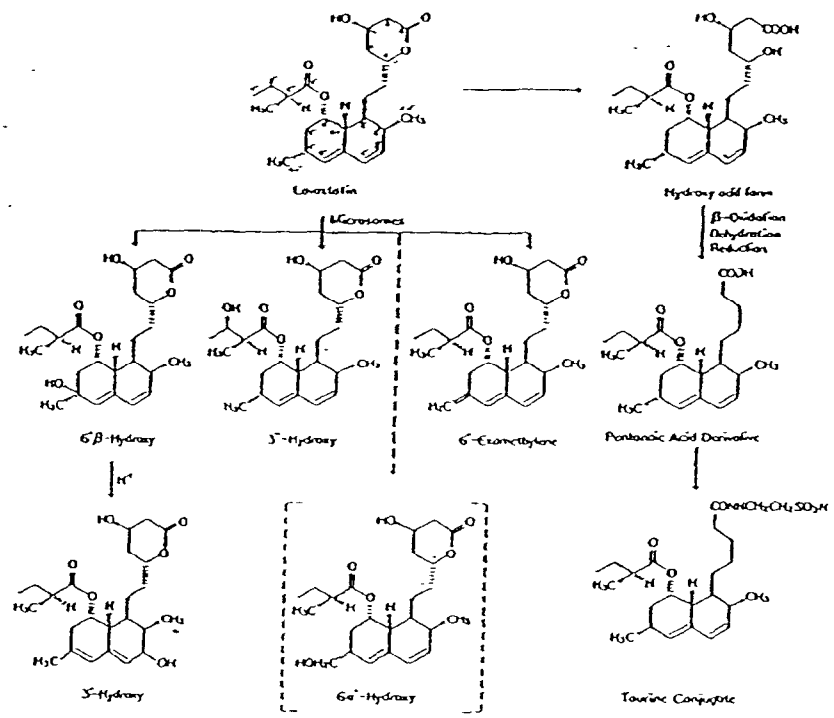
CHEMISTRY

Lovastatin and simvastatin are inactive lactone prodrugs, which after oral ingestion, are hydrolyzed to their corresponding, biologically active, beta-hydroxy acid forms. Pravastatin is marketed as the active beta-hydroxy acid form. Biotransformation of these drug products to several active and inactive metabolites has been reported (Vyas, et al., 1990a; Vyas, et al., 1990b; Halpin, et al., 1993) (Fig. 1). The 6a'-hydroxy-epi-lovastatin, an *in vivo* metabolite found in human and dog plasma, was not detected as a metabolite of rat or mouse liver microsomes. The inactive pentanoic acid derivative, a major metabolite resulting from beta-oxidation of the hydroxy acid form of lovastatin, has been detected in mice and rats; however, it has not been identified as a metabolite in humans.

Lovastatin, simvastatin and pravastatin are competitive inhibitors of HMG-CoA reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of isoprenoid compounds that are intermediates in multiple biosynthetic pathways for biological molecules, including cholesterol, associated with numerous critical organism functions (Brown, et al., 1980) (Fig. 2)

Figure 1.

Biotransformation pathway of lovastatin
(Vyas et al. 1990)



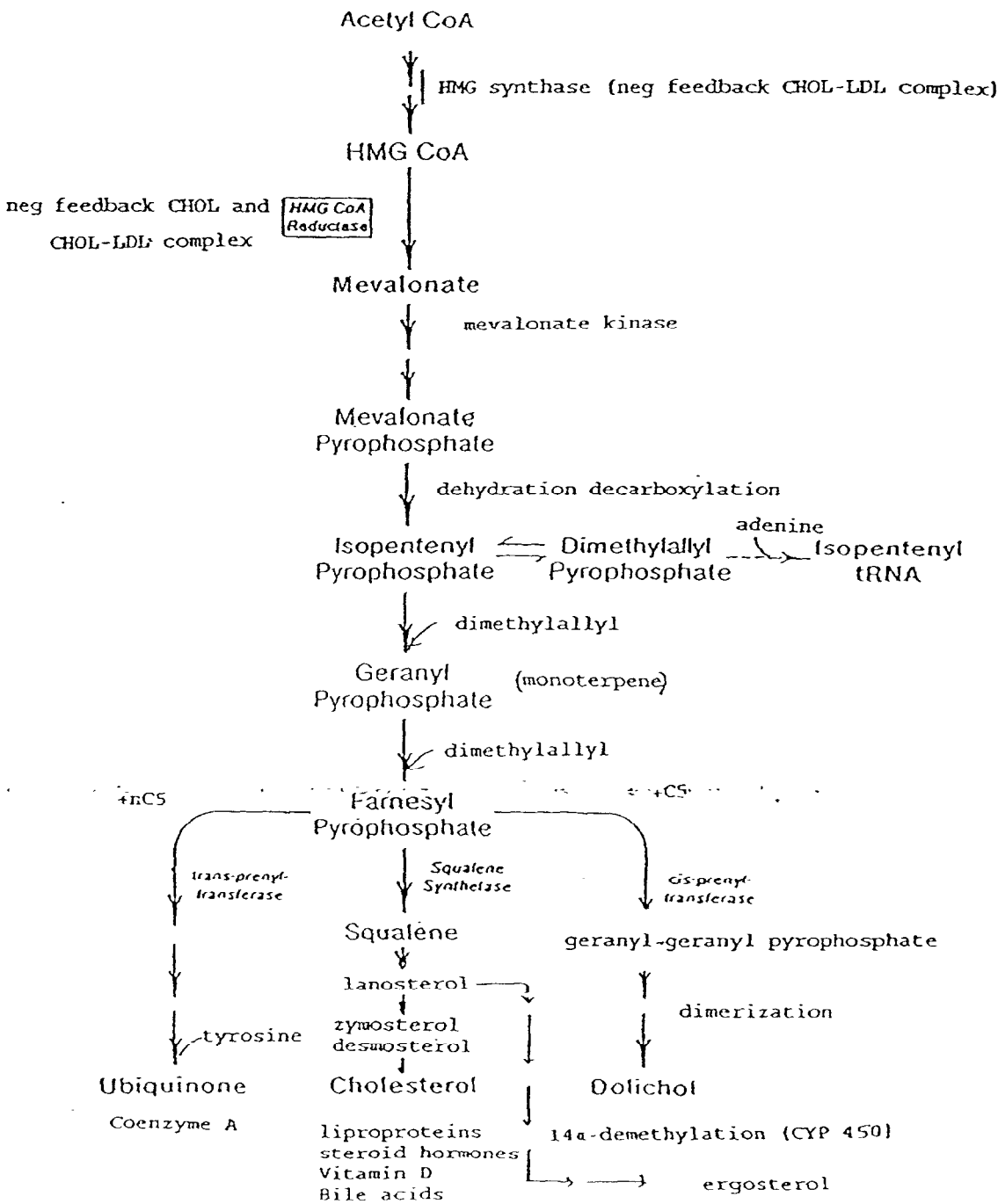
Enzyme-inhibitory activity of lovastatin and its metabolites

Compound	Relative activity[a]	
	Before hydrolysis	After hydrolysis
Lovastatin	0	100
Hydroxy acid form	100	100
6′-β-Hydroxy	0	60
3′′-Hydroxy	0	15
6′-Exomethylene	0	50
3′-Hydroxy	0	0
Taurine conjugate	0	0
Pentanoic acid derivative	0	0

[a] Inhibitory activity of metabolites is expressed relative to the hydroxy acid form of lovastatin.

Figure 2.

Branched Pathway of Mevalonate Metabolism
Modification of Brown et al. 1980



PHARMACOLOGY

Human pharmacokinetics of HMG-CoA reductase inhibitors in plasma have been reviewed by Desager, et al., 1996. Tables I and II from their publication, showing data from multiple published studies, are reproduced below. These data are relevant in that they provide some insight into the drug concentrations to be considered when determining if the definition of "... inhibits in dilute solution ..." has been met. For lovastatin, the recommended dosing range for the treatment of hypercholesteremia is 10-80 mg/day in single or two divided doses; the maximum recommended dose is 80 mg/day. For simvastatin, the recommended dosing range is 5-40 mg/day as a single dose in the evening; the maximum recommended dose is 40 mg/day.

Table I. Main pharmacokinetic parameters of lovastatin (mean ± standard deviation)

Therapeutic daily dose (mg)	C_{max} (ugEq/L)		T_{max} (hours)		AUC (ugEq/L h)[0-24h]		CL/F (L/h)*	
	AI	TI	AI	TI	AI	TI	AI	TI
80 (17 days)	40.7 ± 5.9	49.6 ± 8.3	2.0 ± 0.9	3.1 ± 2.9	305.2 ± 115.7	385.3 ± 107.3	262.1	207.6
80 (single dose-^{14}C)	70.7 ± 61.2	150.6 ± 106.9	2.3 ± 1.3	1.9 ± 1.2	282.4 ± 138.3	570.2 ± 275.4	283.3	140.3
40 (5 days)	45.5 ± 31.5	85.1 ± 58.2	2.41 ± 1	1.8 ± 1.4	236.0 ± 132.3[b]	359.1 ± 205.1	169.5	111.4
40 (7 days)	33.0 ± 9.8	65.7 ± 30.0	2.6 ± 1.3	2.3 ± 1.3	176.9 ± 72.4	284.6 ± 110.5	226.1	140.5
40 (single dose)	9.5 ± 5.2	19.9 ± 8.0	2.9 ± 1.7	2.6 ± 1.7	61.1 ± 72.0	114.1 ± 87	654.7	350.6
20 (single dose)	14.5 ± 8.8	27.1 ± 15.6	2.4 ± 1.2	2.1 ± 1.2	76.3 ± 41.6	114.2 ± 57.6	262.1	175.1

*Calculated from mean values.
[b]0 to 12 hours
Abbreviations: AI = active inhibitors; AUC = area under the plasma concentration-time curve from zero to 24 hours; CL/F = apparent total body clearance; ugEq = ug equivalent; TI = total inhibitors; T_{max} = time to reach peak concentration after drug administration.

Table II. Main pharmacokinetic parameters of simvastatin (mean ± standard deviation)

Therapeutic daily dose (mg)	C_{max} (ugEq/L)		T_{max} (hours)		AUC (ugEq/L h)[0-24h]		CL/F (L/h)*	
	AI	TI	AI	TI	AI	TI	AI	TI
40 (17 days)	45.8 ± 19.5	56.5 ± 24.7	1.4 ± 1.0	1.4 ± 1.0	130.0 ± 32.0	172.0 ± 49.0	307.7	232.5
40 (single dose)	10.3 ± 6.9	34.5 ± 17.3	2.5 ± 1.7	2.3 ± 1.4	40.8 ± 26.3	102.5 ± 45.0	980.4	390.2
20 (single dose)		18.4 ± 7.3		1.7 ± 1.0		61.9 ± 20.6		323.1
100 (single dose - ^{14}C)[b]		125.0 ± 80.0		3.0		1020		98.0
20 (single dose)	9.9 ± 3.4		2.1 ± 1.3		39.6 ± 26.2		505	

*Calculated from the mean values.
[b]Patient with T-tube drainage.
Abbreviations: see table I above.

Other parameters of interest include protein binding effects, adsorption, total body tissue distribution, excretion, and half-life of lovastatin and simvastatin. In plasma, the hydroxy acid and lactone forms of lovastatin are 96 and 98.5% protein bound, respectively. For simvastatin, protein binding for these forms is 98 and 94.5%, respectively. Adsorption for lovastatin and simvastatin is approximately 31% and 60%, respectively. After absorption, these drugs undergo extensive first pass extraction by the liver, their primary site of action. The hydroxy acid form is less efficiently extracted

by the liver than the lactone. The metabolism of lovastatin and simvastatin by the liver is a permanent dynamic process because of the reversibility of the lactone to beta-hydroxy acid reaction. Thus, at any given time, lovastatin will be represented in tissue both as an active hydroxy acid form and as an inactive lactone form. For this reason, publications showing pharmacokinetics data often report "statin" drug concentrations measured as ugEquivalents/ml plasma rather than as ug/ml.

The excretion of inactive metabolites of lovastatin and simvastatin is mainly in feces (64% to 83%) and in urine (10% to 20%). The plasma $t_{1/2\beta}$ ranges from 3 to 4 hours. The pharmacokinetic half-lives are substantially less than the pharmacodynamic values, which are around 20 hours. The apparent total body clearance (CL/F) is very high due to the important first-pass liver extraction effect. Information concerning drug concentrations in other human tissues is limited in the literature and much of our information concerning tissue concentrations of drug are derived from animal studies. Duggan, et al., 1989, have evaluated lovastatin concentrations in numerous tissues of the rat and dog as shown in the table below.

Table 4 from Duggan et al., 1989.

Tissue distribution of lovastatin equivalents in rats and dogs

All values are ug equivalents per g (ml) of tissue. for rats, N = 3; for dogs, N = 4.

Tissue	Rat						Dog, po (60 mg/kg)
	iv (0.8mg/kg)			po (8 mg/kg)			
	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr	4 hr
Plasma	0.23 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.28 ± 0.04	0.27 ± 0.05	0.1 ± 0.01	0.27 ± 0.1
Heart	0.21 ± 0.05	0.03 ± 0.01	<0.02	0.48	0.2	<0.2	0.27 ± <0.1
Lung	0.23 ± 0.04	0.07 ± 0.02	<0.02	<0.5	<0.2	<0.2	0.41 ± 0.2
Liver	2.62 ± 0.83	0.62 ± 0.1	0.15 ± 0.06	6.57 ± 1.13	2.83 ± 0.9	1.01 ± 0.18	4.36 ± 2.0
Spleen	0.08 ± 0.02	0.04 ± 0.01	<0.02	<0.1	0.18 ± 0.06	<0.2	NS*
Adrenal	<0.28	<0.19	NS	<0.4	0.60	0.56 ± 0.05	0.22 ± 0.12
Kidney	0.39 ± 0.07	0.13 ± 0.02	0.04 ± 0.02	0.58 ± 0.04	0.47 ± 0.09	0.2 ± 0.03	0.71 ± 0.26
Stomach	0.12 ± 0.02	0.05 ± 0.01	0.02 ± 0.01	7.59 ± 5.02	12.16 ± 4.42	0.3 ± 0.08	NS
Small Intestine	2.52 ± 0.19	0.54 ± 0.08	0.07 ± 0.02	17.25 ± 5.51	11.26 ± 5.42	0.49 ± 0.13	13.62 ± 9.35
Large Intestine	0.14 ± 0.01	0.84 ± 0.23	0.05 ± 0.01	0.02 ± 0.07	5.2 ± 1.07	0.65 ± 0.48	NS
Testes	0.16 ± 0.01	0.03 ± 0.01	<0.02	<0.1	0.09 ± 0.01	0.04 ± 0.01	0.21 ± 0.02
Muscle	0.09 ± 0.03	<0.02	<0.02	<0.1	0.12	<0.1	0.35 ± 0.15
Fat	0.11 ± 0.02	0.02 ± 0	0.02	<0.1	0.12	0.29	NS
Brain	0.06 ± 0	0.02 ± 0	<0.03	0.1	0.08 ± 0.02	0.05 ± 0.01	0.17 ± <0.1

*NS, not significant.

The data from the above tables (Tables 1 and 2) would suggest that a maximum approved human dose of either lovastatin or simvastatin, administered chronically on a daily basis, would be expected to result in a C_{max} plasma drug concentration of approximately 40-60 ugEq/L (~0.1 uM) at steady state conditions. With a t_{max} occurring at 2-3 h and a $t_{1/2\beta}$ of 3 to 4 h, the trough plasma drug concentrations evident within 8 to 11 h would be expected to be ≤ 10-15 ugEq/L (~0.025 uM) following once daily oral dosing. Plasma drug concentrations following a single administered dose were slightly higher, but a single dose of drug would not be expected to provide activity of sufficient duration to treat an infectious disease. Interpretation of the data provided for tissue concentrations in rats and dogs treated with lovastatin suggests that with the exception of the liver, stomach, and intestines other body tissues exhibit lovastatin concentrations similar to or less than that observed in plasma. Although the human equivalent dose is different from that administered to these animals, lovastatin in humans is expected to exhibit a similar tissue distribution profile, relative to plasma concentrations, as that shown above for the rat and dog. Therefore, with respect to human clinical use of lovastatin and simvastatin, the target definition for "... inhibits in dilute solution ..." relevant to preclinical studies of antimicrobial activity drug concentrations should lie somewhere between 10 and 60 ugEq/L (~0.025 to 0.1 uM). The lovastatin concentration of 10 ugEq/L would represent that expected during the trough concentration phase while the 60 ugEq/L concentration would represent the upper range of C_{max} reported. However, because the pharmacokinetic half-lives are substantially less than the pharmacodynamic values of approximately 20 h, the trough concentrations may not be relevant to antimicrobial activity evaluation. Consequently, the C_{max} concentration of ~0.1 uM may represent a better choice for relevant comparisons to be made

MICROBIOLOGY

Publications containing data relevant to possible antimicrobial activity of lovastatin and related "statin" drugs are presented below. The publications were evaluated and summarized independently to ensure that potentially critical parameters pertaining to each study were not co-mingled. A complete citation for each reference summarized is provided in bold type. Activity data are grouped by microorganism classification for ease of reference. A general summary of the data is provided in Tables 1-4 in the discussion section at the end of this report.

Lovastatin Antimicrobial Activity Against Bacteria:

Zhou, D., et al. Early steps of isoprenoid biosynthesis in Escherichia coli. Biochem J. 1991 Feb 1; 273(Pt 3): 627-34.

In this paper the authors reported the lack of involvement of mevalonic acid in the early steps of isoprenoid biosynthesis in *E. coli*. Mevinolin (lovastatin) at concentrations as high as 68.3 uM did not affect growth of *E. coli*. Interpretation of data presented in this paper (while not ruling out involvement of non-membrane bound mevalonate) would suggest that eubacteria, such as *E. coli*, do not utilize acetyl-CoA and mevalonic acid in the biosynthesis of isoprenoids as has been reported for archaebacteria and eukaryotes (*vide infra*). The authors suggested that if the alternative pathway for biosynthesis of isoprenoids in *E. coli* is a general characteristic of all eubacteria, then it may represent a clear biochemical marker that separates eubacteria from archaebacteria and eukaryotes. If true, inhibitors of HMG-CoA reductase, such as lovastatin, would not be expected to inhibit growth of species of true bacteria. Data showing lovastatin growth inhibition of any bacteria other than those classified as archaebacteria were not found in the published literature.

Cabrera, JA., et al. Isoprenoid synthesis in Halobacterium holobium. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A concentration in response to mevalonate availability. J Biol Chem. 1986 Mar 15; 261(8): 3578-83.

In this paper, the authors utilized *H. holobium*, a genus of organism representative of those archaebacteria which require >15% NaCl for growth [Dundas, I.E.D. (1977) Adv. Microb. Physiol. 15, 85-120; cited in Cabrera, et al., 1986], as a unique biological model to study the regulation of mevalonate synthesis. They reported data which supports the conclusion that *H. holobium*'s HMG-CoA concentration, and not HMG-CoA reductase activity, was reversibly modulated in response to mevalonate availability, in contrast that reported for eukaryotic cells. As part of their experimental design, they evaluated mevinolin (lovastatin) induced effects on mevalonate content of *H. holobium*. They demonstrated that growth of *H. holobium* was completely inhibited by mevinolin (lovastatin) at concentrations of 1-2 uM (~0.4 to 0.8 ug/ml). This inhibition by lovastatin was reversed by the addition of 4 mM mevalonate to the culture medium.

Lam, WL., et al. Shuttle vectors for the archaebacterium Halobacterium volcanii. Proc Natl Acad Sci USA. 1989 Jul; 86(14): 5478-82.

In this publication, the authors reported that lovastatin completely inhibited *in vitro* growth of the *Archaebacterium, H. volcanii* strain WFD11, at 1-2 uM (~0.4 to 0.8 ug/ml) and at 20-40 uM (~8 to 16 ug/ml) when cells were grown on agar prepared with minimal or enriched medium, respectively. The differential sensitivity of microorganisms to lovastatin inhibition when grown on medium with and without lipids is a commonly reported observation in the published literature.

Lovastatin Antimicrobial Activity Against Viruses:

Overmeyer, JH. Isoprenoid requirement for intracellular transport and processing of murine leukemia virus envelope protein. J Biol Chem. 1992 Nov 5; 267(31): 22686-92.

In this publication, the authors examined the potential relationship between isoprenoid biosynthesis and the processing of murine leukemia virus (MuLV) envelope glycoprotein in murine erythroleukemia (MEL) cells cultured in Dulbecco's Minimum Essential Medium (MEM) with 10% fetal bovine serum. They reported that lovastatin, at concentrations as low as 1 ug/ml (~2.5 uM), was not cytostatic for MEL cells in culture, but prevented the cells' ability to convert MuLV envelope glycoprotein precursor, $gPr90^{env}$, to the mature envelope glycoprotein, $gp70^{env}$. This conversion normally occurs within the Golgi apparatus. It was suggested that lovastatin may prevent viral envelope precursors from reaching the Golgi compartment by blocking the geranylgeranyl isoprenylation of the GTP-binding *rab* proteins required for the transport of precursor viral glycoprotein from the endoplasmic reticulum (ER) to the Golgi apparatus. In cells infected with retroviruses, the envelope glycoproteins encoded by the viral *env* genes normally undergo proteolytic processing and oligosaccharide maturation upon translocation from the ER to the Golgi apparatus. Inhibition of proteolytic cleavage of viral envelope proteins is known to reduce infectious virus titers. The authors reported that the lovastatin inhibitory effect on envelope maturation was drug dose dependent and was completely reversed by the addition of 200 uM mevalonate to the culture medium. However, the authors did not report the effect, if any, of lovastatin on MuLV infectivity.

Maziere, JC., et al. Lovastatin inhibits HIV-1 expression in H9 human T lymphocytes cultured in cholesterol-poor medium. Biomed Pharmacother. 1994; 48(2): 63-7.

In this publication, the authors investigated the *in vitro* effect of lovastatin on HIV production in H9 T lymphocytes adapted to grow in RPMI 1640 medium supplemented with only 1% human serum to limit exogenous cholesterol supply. Lovastatin (0.3 uM final concentration) (~0.12 ug/ml) was added to the culture medium 1 day post-infection. The medium was replaced each day by new medium containing the same concentration of lovastatin. Reverse transcriptase activity was reduced approximately 10-fold after 9 days of lovastatin treatment compared to untreated, infected controls. The authors concluded from these data that clinical intervention that would lower cholesterol availability for HIV viral membrane synthesis may have some benefit in treatment of viral replication in human AIDS patients. The effects of adding additional exogenous cholesterol or serum on the observed antiviral activity was not investigated.

Malvoisin, E., et al. Effect of drugs which inhibit cholesterol synthesis on syncytia formation in vero cells infected with measles virus. Biochim Biophys Acta. 1990 Feb 23; 1042(3): 359-64.

For these studies, Vero cells were infected with measles virus (Hallé strain) and incubated in Eagle's minimum essential medium containing 2% fetal calf serum and antibiotics (100 units/ml penicillin and 100 ug/ml streptomycin). Inhibitors of cholesterol biosynthesis [including mevinolin at 6 ug/ml (~15 uM)] inhibited measles virus induced syncytia in Vero cells, but this effect was not necessarily related to an inhibition of virus infectivity. Inhibition of virus infection occurred with some non-statin cholesterol synthesis inhibitors, but appeared to be due to the inhibitor's effects on parameters other than cholesterol synthesis. Inhibition of virus infection by mevinolin was not reported. Furthermore, cell cytotoxicity related to mevinolin was not reported. Thus, although mevinolin significantly reduced syncytia formation in measles virus infected Vero cells, antiviral activity was not reported to be associated with this effect.

Lovastatin Antimicrobial Activity Against Yeast and Fungi:

Ikeura, R., et al. Growth inhibition of yeast by compactin (ML-236B) analogues. J Antibiot Tokyo. 1988 Aug; 41(8): 1148-50.

In this publication, a variety of HMG-CoA reductase inhibitors, including lovastatin (monacolin K), were evaluated for antimicrobial activity against 303 strains of yeast representing 41 genera and 165 species. All of the HMG-CoA reductase inhibitors were converted to their respective active hydroxy acid form by hydrolysis prior to use.

Yeast strains were inoculated onto 0.67% yeast nitrogen base medium containing 0.5% glucose and 1.5% agar (pH 5.3), and grown at 30 C. Where indicated, compactin (lovastatin is an analogue of compactin) was supplemented to the medium at a concentration of 0-20 ug/ml (~0-50 uM). Growth was inspected after 4 days of cultivation. The authors stated that of 303 strains tested, 43 strains (18 genera, 35 species), 21 strains (13 genera, 19 species) and 4 strains gave no detectable growth on the agar medium containing 20, 10 and 4 ug/ml of compactin, respectively (50, 16, and 10 uM, respectively). The remaining 260 strains (34 genera, 135 species) were resistant to compactin at 20 ug/ml (~50 uM), data not shown. The most sensitive 4 strains were *Rhodotorula glutinis* H3-9-1, *Sporobolomyces salmonicolor* WF 188, *Aessosporon salmonicolor* IFO 1845 and *Citeromyces matritensis* IFO 0954 with MIC values of 0.1, 1.0, 2.0 and 2.0 ug/ml, respectively (range ~0.25 to 5 uM). The identity of the remaining 299 strains was not reported.

Growth inhibition was subsequently determined for *R. glutinis* H3-9-1 and *S. salmonicolor* WF 188 in liquid medium consisting of 0.67% yeast nitrogen base and 0.5% glucose. Inhibitors were added at concentrations of 0-100 ug/ml (~0 to 250 uM) and cells were cultured with shaking at 30 C for 4 days. Growth was monitored by measuring OD at 550 nm. Monacolin K (lovastatin) and compactin were the most potent inhibitors having MIC values of 0.1 and 1.0 ug/ml (~0.25 and ~2.5 uM) for *R. glutinis* H3-9-1 and *S. salmonicolor* WF 188, respectively. Inhibitory activity of the other HMG-CoA reductase inhibitors (ML-236A, monacolin L, and monacolin X) were 1/25 - 1/50 of the above values.

In a separate experiment, the ability of mevalonate to reverse the inhibition of compactin against the 4 most sensitive strains mentioned above was evaluated in a dose dependent study. At 10 mM, mevalonate completely reversed the compactin inhibition for all strains except for *Citeromyces matritensis* IFO 0954. However, the growth curve for *C. matritensis* IFO 0954 in the absence of compactin was substantially reduced when compared to the growth curves of the other strains grown under the same conditions. This observation suggests that under normal culture conditions, growth of *C. matritensis* IFO 0954 was aberrant and compactin inhibition was substantially more detrimental under these circumstances. Thus, the relevance of the inhibition pattern for *C. matritensis* is difficult to interpret.

Lorenz, RT., et al. Effects of lovastatin (mevinolin) on sterol levels and on activity of azoles in Saccharomyces cerevisiae. Antimicrob Agents Chemother. 1990 Sep; 34(9): 1660-5.

In this publication, the authors reported the quantitative effects of lovastatin on the free sterol and steryl ester fractions of wild type *Saccharomyces cerevisiae*, strain 2180-1A. In these studies, the organisms were grown in medium (YPD) consisting of 2% glucose, 1% peptone, and 1% yeast extract. Minimal inhibitory concentrations (MICs) were determined by inoculating 5 ul of an overnight culture into YPD medium and incubating at 28° C with constant shaking. The MICs were recorded as the lowest concentration of antifungal agent at which no significant visible growth occurred after 3 days. Lovastatin lactone prodrug that was used in this study was hydrolyzed to the active hydroxy acid form prior to use.

Lovastatin at 10 ug/ml (~25 uM) was reported to dramatically decrease the total endogenous steryl ester fraction in *S. cerevisiae*. As the concentration of lovastatin increased progressively above 10 ug/ml, the free sterol fraction decreased linearly. Moreover, in addition to severely decreasing the accumulation of endogenous steryl esters , lovastatin prevented the esterification of sterol taken up from the medium. However, the growth rate and cell yield were not significantly affected until a lovastatin concentration of 75 ug/ml (~190 uM) or greater was present in the medium; at concentrations above 150 ug/ml (~380 uM), the growth rate and cell yield were severely diminished (data not shown).

In combination studies, *S. cerevisiae* was grown with different amounts of lovastatin and ketoconazole, clotrimazole or miconazole. Interpretation of the results obtained indicated that there was a synergistic effect of lovastatin and different azoles in lowering the MICs of azole antifungal agents. Lovastatin at 2 ug/ml (~5 uM)significantly decreased the MICs of each azole. In the presence of lovastatin at 10 ug/ml (~25 uM), the MICs of clotrimazole, ketoconazole, and miconazole were decreased 6-, 10-, and 32-fold, respectively. The authors hypothesized that the synergism observed between lovastatin and these azoles may be due to increased cell membrane permeability caused by the effect of lovastatin on the sterol content of the organism. The authors reported that *S. cerevisiae* cell membrane permeability to

exogenous sterols was increased under conditions where endogenous sterols were decreased (see above). They speculated that as membrane permeability was increased for sterols then it may be increased for other agents, such as azoles, as well. However, data were not provided to demonstrate that intracellular concentrations of azoles occurred under these conditions.

Sud, IJ., et al. Effect of ketoconazole in combination with other inhibitors of sterol synthesis on fungal growth. Antimicrob Agents Chemother. 1985. 28: 532-534.

The authors of this publication evaluated, *in vitro*, the inhibitory effects of ketoconazole, mevinolin (lovastatin) and a combination of these two drugs against a variety of fungi. The data below are taken from Tables 1 and 2 of their publication.

Sterol synthesis inhibitors

Fungus tested	MIC (ug/ml) of inhibitor		Concn (ug/ml) of inhibitor giving a fourfold or greater decrease in the MICs of ketoconazole
	Ketoconazole	Mevinolin	Mevinolin
Candida albicans VA	0.045	50	3.12 (4)[a]
Candida albicans 7.22	3.12	100	25.0 (8)
Candida tropicalis	0.78	>100	--[b]
Torulopsis glabrata	0.78	>100	--
Aspergillus fumigatus 173	3.12	6.25	3.12 (8)
Aspergillus fumigatus	6.25	6.25	0.78 (4)
Aspergillus niger	12.5	12.5	0.78 (4)[c]
			1.56 (8)
Rhizopus rhizopodiformis	6.25	50	12.5 (4)
			25.0 (8)

[a]Numbers in parentheses represent the fold decrease in the MIC of ketoconazole in the presence of the indicated concentrations of Mevinolin.
[b]No change or less than a fourfold decrease in the MIC of ketoconazole in the presence of mevinolin.
[c]Where more than one number is given, the lower number is the concentration of the drug giving a fourfold decrease in the MIC of ketoconazole, and the higher number is the concentration showing the maximum effect.

These data were generated in *in vitro* studies utilizing completely synthetic media. The species most sensitive to mevinolin (lovastatin) were *A. fumigatus* and *A. niger* with MICs of 6.25 and 12.5 ug/ml (~16 uM and ~32 uM), respectively. These species were also the ones showing the most sensitivity to the combination effects (4- to 8-fold decrease in MICs of ketoconazole) of ketoconazole and lovastatin. The ability of intermediates of the isoprenoid and steroid pathways, subsequent to mevalonic acid synthesis, to reverse the inhibitory effects of lovastatin observed in this study was not evaluated.

Bejarano, ER., et al. Independence of the carotene and sterol pathways of Phycomyces. FEBS Lett. 1992 Jul 20; 306(2-3): 209-12.

In this publication, the authors evaluated the pathway for the synthesis of carotene and sterols in *Phycomyces blakesleeanus* and various mutants with altered carotenogenesis. The fungus was grown on minimal agar medium at 22° C in the dark. Lovastatin and mevalonic acid lactone were hydrolyzed to the hydroxy acid forms prior to addition to growth medium. *Phycomyces* did not grow on medium with 1 uM (~0.4 ug/ml) lovastatin. This inhibition was reversed by the presence of mevalonate in the medium at 10 mM, but not at 1 mM.

Engstrom, W., et al. The effects of tunicamycin, mevinolin and mevalonic acid on HMG-CoA reductase activity and nuclear division in the myxomycete Physarum polycephalum. J Cell Sci. 1989 Mar; 92(Pt 3): 341-4.

In this publication, the authors reported that lovastatin at concentrations ≥ 25 uM (~10 ug/ml), inhibited protein synthesis, DNA synthesis, nuclear division and plasmodia growth, *in vitro*, of *Physarum polycephalum*. These effects could be partially reversed by the addition of mevalonate at concentrations ≥ 0.4 mM.

Lovastatin Antimicrobial Activity Against Parasites:

Andersson, M., et al. Lovastatin inhibits interferon-gamma-induced Trypanosoma brucei proliferation: evidence for mevalonate pathway involvement. J Interferon Cytokine Res. 1996 Jun; 16(6): 435-9.

In this publication, the authors reported that interferon-gamma, at low concentrations (10^3 U/ml added to 10^6 parasites), had a growth stimulatory effect on *Trypanosoma brucei brucei in vitro* and that this proliferative response was blocked by low levels of lovastatin (0.1 uM) (~.04 ug/ml). However, lovastatin did not inhibit growth at concentrations as high as 20 uM (~8 ug/ml), the highest concentration tested, when added to nonstimulated cultures of the parasite.

> Note: In this study, lovastatin concentration was given as uM in the figures, but was given as mM in the figure legends and in the text of the paper. Lovastatin is insoluble in water (Mevacor package insert). Therefore, it is assumed, but not known with certainty, that the values listed as uM were the correct concentrations to use in this report.

Florin-Christensen, M., et al. Inhibition of Trypanosoma cruzi growth and sterol biosynthesis by lovastatin. Biochem Biophys Res Commun. 1990 Feb 14; 166(3): 1441-5.

In this publication, the authors report a dose dependent lovastatin inhibition of the *in vitro* growth of *Trypanosoma cruzi* epimastigotes at 10 and 30 ug/ml (~25 and ~75 uM, respectively). Squalene at 100 uM, but not cholesterol, reversed lovastatin's growth inhibitory effects induced by 10 and 30 ug/ml suggesting that lovastatin interfered with steps leading to squalene biosynthesis. At 50 ug/ml (~125 uM), lovastatin killed most of the trypanosomes. Squalene was not able to reverse the inhibitory effects on epimastigotes treated with 50 ug/ml of lovastatin.

Haughan, PA., et al. Synergism in vitro of lovastatin and miconazole as anti-leishmanial agents. Biochem Pharmacol. 1992 Dec 1; 44(11): 2199-206.

In this publication, the authors reported on the *in vitro* combinational use of the antifungal drug, miconazole, with the cholesterol lowering drug, lovastatin, to assess their potency as anti-leishmanial agents. Activity was assessed for each drug as single agents and in combination against Leishmania promastigotes and amastigotes.

Lovastatin, as a single drug, had IC_{50} values of 82 ug/ml (~200 uM) and 20 ug/ml (~50 uM) against *L. donovani* and *L. amazonensis* promastigotes, respectively. Miconazole, as a single drug, had IC_{50} values of 6 and 3 ug/ml, respectively, against these life-cycle forms. Treatment of *L. amazonensis* amastigotes in mouse peritoneal macrophages with lovastatin up to a concentration of 10 ug/ml (~25 uM) had little effect on the percentage of macrophages infected or the number of amastigotes in the macrophages. Due to drug insolubility problems, and IC_{50} could not be determined, but it was estimated to be well in excess of 10 ug/ml (~25 uM). The IC_{50} for miconazole was estimated to be 8 ug/ml.

When used in combination, miconazole and lovastatin IC_{50} concentrations of each drug could be reduced by 2- to 10-fold, suggesting a synergistic activity interaction against these life-cycle forms of these *Leishmania spp.*

Morrison, DD., et al. Effects of steroids and steroid synthesis inhibitors on fecundity of S. mansoni in vitro. J Chem Ecol. 1986; 12: 1901-08.

Mevinolin (lovastatin) was reported to significantly depress egg production (~50%) at 1 uM (0.4 ug/ml) in *Schistosoma mansoni* grown *in vitro* for 72 h at 37°C with shaking in medium that was a 1:1 mixture of RPMI 1640 and heat-inactivated horse serum, adjusted to pH 7.4. Penicillin and streptomycin (100 ug/ml each) were added along with mercaptoethanol to a final concentration of 5×10^{-5} M. Mevinolin at higher concentrations (i.e., 10 uM and 100 uM) (~4 and ~40 ug/ml, respectively) was unable to completely inhibit egg production. Effects on adult mating pairs appeared to be minimal even at 100 uM lovastatin. Adult schistosomes are incapable of *de novo* cholesterol formation [(Meyer et al., 1970; Smith et al., 1970); cited by the authors of this paper]. Egg production inhibition by lovastatin was not reversed by coincubation with 100 uM cholesterol. Morrison, et al., concluded from these data that lovastatin inhibition of egg production is not due to a steroid-mediated effect.

Vandewaa, EA., et al. Physiological role of HMG-CoA reductase in regulating egg production by Schistosoma mansoni. Am J Physiol 1989 Sep; 257(3 Pt 2): R618-25.

The purpose of this publication was to provide evidence suggesting that HMG-CoA reductase activity plays a critical role in parasite egg production. Several lines of evidence, described below, were provided to support this hypothesis.

White outbred (ICR) female mice, infected intraperitoneally with 250-300 schistosome cercariae, were dosed daily with lovastatin (50 or 250 mg/kg) by gavage for 3 days starting at 42 days postinfection. Control mice were dosed with vehicle only. After treatment of these acutely infected mice, parasites were collected and microsomes were prepared. HMG-CoA reductase enzyme activity measured in microsomes obtained from schistosomes exposed to 250 mg/kg lovastatin was reduced significantly (~3-fold) compared to untreated controls. However, if the lovastatin exposed parasites were subsequently grown *in vitro* for 24 h in drug free medium prior to assay for HMG-CoA reductase activity, the enzyme activity was observed to be significantly enhanced (~2-fold) over controls. In contrast to these results, parasites collected from mice treated with 50 mg/kg lovastatin were shown to have a significant induction in HMG-CoA reductase activity over controls.

Because low doses of lovastatin (50 mg/kg vs. 250 mg/kg) produced higher levels of HMG-CoA reductase activity in the above experiments, egg production in schistosomes obtained from lovastatin treated mice, dosed daily for 10 days at 50 mg/kg starting at 35 days postinfection, was evaluated *in vitro* (Table 2 from Vandewaa, et al., 1989).

Table 2. Effect of mevinolin on *in vitro* egg production by *S. mansoni* after *in vivo* exposure to the drug

In Vivo Treatment	Concentration of Mevinolin in Culture Media	No. Eggs
Vehicle	0	60.4 ± 32.6
Vehicle	10 uM	10.7 ± 7.3*
Mevinolin (50 mg/kg)	0	321.8 ± 90.4*
Mevinolin (50 mg/kg)	10 uM	6.3 ± 4.1*

Data are means ± SD for number of eggs per female per 72 h. Parasites were incubated in the presence or absence of mevinolin following *in vivo* exposure to the drug or its vehicle. *Significantly different from control, $P < 0.01$.

These results show that adult *S. mansoni* schistosome egg production, measured in an *in vitro* assay, was stimulated approximately 5-fold in infected mice treated with 50 mg/kg lovastatin. Moreover, this stimulation could be blocked

upon the addition of 10 uM mevinolin to the *in vitro* culture medium. Furthermore, it was reported that lovastatin's *in vitro* inhibition of schistosome egg production could be reversed by the addition of either farnesol or mevalonate at a concentration of 80 uM. These data, taken together with the fact that schistosomes are incapable of synthesizing cholesterol *de novo*, led these authors to conclude that a nonsterol lipid, yet to be identified, may play an important role in regulating egg production by *S. mansoni*.

These *in vitro* observations led to experiments wherein *in vivo* egg production by schistosomes was measured in mice treated with 50 or 250 mg/kg lovastatin. Drug was administered by oral gavage for 10 days beginning 35 days postinfection. The results on *in vivo* egg production correlated with observations on HMG-CoA reductase enzyme activity and on *in vitro* egg production, mentioned above. At 50 mg/kg, egg production, *in vivo*, was enhanced over that observed in control animals (degree of enhancement not reported). In mice treated with 250 mg/kg, egg production was inhibited 45.4% compared to control animals. This reduction in egg production was correlated with a reduction in liver pathology associated with schistosome infections in mice. Reduction in pathology did not occur in infected mice treated with lovastatin at 50 or 100 mg/kg. Adult worm burden was unaffected by treatment with lovastatin at any of the concentrations evaluated.

From these studies, the authors concluded that "... Although the chronic application of mevinolin to an infected human would be an inappropriate strategy for the control of the disease associated with the infection, we felt that the consequences of a continuous application of mevinolin to infected mice should validate the concept that a reduction in egg production should reduce the parasite-induced pathology."

Chen, GZ., et al. Antischistosomal action of mevinolin: evidence that 3-hydroxy-methylglutaryl-coenzyme A reductase activity in Schistosoma mansoni is vital for parasite survival. Naunyn Schmiedebergs Arch Pharmacol. 1990 Oct; 342(4): 477-82.

This publication is an extension of the observations reported by Vandewaa, et al., 1989, described above. In this paper, these authors reported on adult and developing schistosome survival in mice administered 0.2% lovastatin (equivalent to 640 mg/kg/day) in the diet for 14 days, beginning 35 to 45 days postinfection. Results from this study show that 96-100% of adult parasites were eliminated by this treatment. These effects were shown to be drug dose dependent. Administration of the same dose beginning 2 days prior and continuing for 15 days after infection (juvenile stage of parasite growth), resulted in 93-96% reduction of adult parasites. To determine if lovastatin could be shown to be lethal in *in vitro* cultures of schistosomes, adult parasites were exposed to increasing doses of lovastatin (1 to 10 uM). Lactate production and motility in these treated parasites, as a measure of drug toxicity, were observed over time. The response was time and dose dependent. At 3 days incubation, 10 uM lovastatin reduced motility and lactate production > 50%, at 11 days of culture, doses of 1-10 uM inhibited activity nearly 90%. It was stated that inhibition of motility and lactate production eventually resulted in death of the organism but it was not clear from the results provided as to when death would actually occur.

Urbina, JA., et al. Mevinolin (lovastatin) potentiates the antiproliferative effects of ketoconazole and terbinafine against Trypanosoma (Schizotrypanum) cruzi: in vitro and in vivo studies. Antimicrob Agents Chemother. 1993 Mar; 37(3): 580-91.

In this study, the authors evaluated the potentiation effect of lovastatin on the antiproliferative effects of ketoconazole and terbinafine against *Trypanosoma cruzi*, the causative agent of American trypanosomiasis (Chagas' disease). Activity against epimastigotes and amastigotes *in vitro* and parasitemia *in vivo* was determined for each single drug and also for the drugs when used in combination. For all *in vitro* studies reported, lovastatin was hydrolyzed to the active hydroxy acid drug form prior to use

In *in vitro* studies, the epimastigote form was cultivated in liver infusion-tryptose medium supplemented with 10% calf serum at 28° C with strong agitation (120 rpm). The antiproliferative effects were measured at various times after addition of varying concentrations of each drug alone and in combination. Results obtained from these *in vitro* studies showed that lovastatin, at 7.5 uM (3 ug/ml), ketoconazole at 0.1 uM and terbinafine at 1 uM, each, reduced growth of *T. cruzi* epimastigotes 20% to 30% when evaluated as single agents. Lovastatin at 50 and 75 uM (20 and 30 ug/ml, respectively) caused complete growth arrest with cell lysis ensuing at 144 and 96 h, respectively. Lovastatin at 7.5 uM in combination with ketoconazole at 0.1 uM resulted in complete growth arrest followed by cell lysis at 144 h. Thus, the authors concluded that the trypanocidal concentration of lovastatin was reduced by a factor of 10 in the presence of a ketoconazole concentration that by itself had only very modest effects on parasite growth. Terbinafine in combination with lovastatin produced a lesser effect, complete growth inhibition and lysis required 25 uM lovastatin with 1 uM terbinafine.

The authors also reported on the effects of lovastatin on *T. cruzi* amastigotes proliferating inside Vero cells *in vitro*. Lovastatin at 1 uM (0.4 ug/ml) produced less than a 30% reduction in the number of parasites per Vero cell and % of infected cells after incubation at 37° C for 96 h. At concentrations greater than 1 uM, lovastatin had a deleterious effect on the host cells; thus, the antiparasitic activity measured is close to the cytotoxic drug concentration for the Vero cells (i.e., the therapeutic index is close to 1). However, lovastatin at 0.75 uM in combination with 1 nM ketoconazole, which by itself produced a 30 to 40% reduction of in the number of infected cells, produced a complete elimination of amastigotes without deleterious effects on the host cells when cells were treated for 192 h. When terbinafine and lovastatin were evaluated in combination, only additive effects on amastigote reduction were observed. In these studies, amastigotes were cultivated in Vero cells maintained in minimal essential medium supplemented with 2% fetal calf serum in humidified 95% air-5% CO_2 atmosphere at 37° C. The medium, with and without drug, was changed every 48 h.

From their *in vivo* murine model of Chagas' disease, the authors reported the following results:

> "... mice treated orally with ketoconazole at 30 mg/kg of body weight per day for 7 days were fully protected from death 40 days after infection with a lethal inoculum of *T. cruzi* blood trypomastigotes, while all the controls (untreated) were dead 24 days postinoculation; ketoconazole at this dose completely suppressed parasitemia. When the dose of ketoconazole was lowered to 15 mg/kg/day, incomplete protection against death and significant numbers of circulating parasites were observed for up to 25 days. Mevinolin at 20 mg/kg/day promoted 50% survival, but the level of parasitemia was comparable to that observed in the controls. However, when the low dose of ketoconazole was combined with mevinolin, 100% survival and almost complete suppression of parasitemia were observed, indicating a synergic action in vivo, which was most evident in the effect on circulating parasites ..."

In these studies, drugs, suspended in 2% methylcellulose containing 0.5% Tween 80, were given by gavage once daily for 7 days. *T. cruzi* Y strain was inoculated (10^3 trypomastigotes) intraperitoneally into female outbred NMRI albino female mice weighing 25 to 30 g and treatment was initiated 24 h later.

Brener, Z., et al. An experimental and clinical assay with ketoconazole in the treatment of Chagas disease. Mem Inst Oswaldo Cruz. 1993 Jan-Mar; 88(1): 149-53.

In this publication the authors tested the *in vivo* activity of ketoconazole associated with lovastatin for possible synergistic activity against *T. cruzi* Y strain infection in mice (see table below). Other drugs evaluated in this publication were not reviewed for this report.

In this study, groups of Swiss albino mice, weighing 18-20 g were inoculated intraperitoneally with 2 x 10^4 blood forms of *T. cruzi* Y strain. Treatment was started 24 h after infection and continued for 20 days. Drugs were prepared in distilled water and administered by oral gavage.

Table II from Brener, et al., 1993.

Parasitemia and mortality in groups of mice inoculated with 2 X 10^4 blood forms of the *Trypanosoma cruzi* Y strain and treated with ketoconazole, lovastatin and association of both drugs

Drug (mg/kg)	No. parasites/5 ul (7^{th} day)	Mortality (20^{th} day)
Ketoconazole (100)	0	0/6
Ketoconazole (40)	780	1/6
Ketoconazole (25)	14.670	1/5
Lovastatin (100)	64.333	6/6
Ketoconazole (40)	689	1/5
Lovastatin (10)		
Ketoconazole (25)	15.480	4/5
Lovastatin (25)		
Untreated controls	19.021	5/5

Note: The data reported in this table is in conflict with statements made by the authors in the text. The numbers reported in the table for the No. parasites/5 ul for Ketoconazole (40) and the combination of Ketoconazole (40) with Lovastatin (10) appear to be incorrect based upon text information provided. Rather than the numbers 780 and 689 as reported above, the actual numbers may be 0.780 and 0.689, respectively. It appears that a decimal proceeding the number was omitted when printed by the publisher. It is not certain that this assumption is valid. However, the analysis of the results has been based upon the written text information which implies that this assumption is reasonable.

Interpretation of data presented in this publication shows that lovastatin at the highest dose evaluated (100 mg/kg) exacerbated parasitemia approximately 3-fold over untreated controls and failed to provide a survival benefit associated with treatment. Ketoconazole at 100 mg/kg eliminated the parasitemia and 100% of the mice survived. Ketoconazole at 25 mg/kg reduced parasitemia approximately 20% and 80% of the mice survived; whereas, all of the untreated controls died. If the assumption that the correct numbers for parasitemia in the groups of mice treated with ketoconazole, as discussed above, are 0.780 and 0.689, then the parasitemia data reported in this table suggest that lovastatin in combination with ketoconazole is antagonistic in this infection model with respect to parasitemia.

Lujan, HD., et al. Isoprenylation of proteins in the protozoan Giardia lamblia. Mol Biochem Parasitol 1995 Jun; 72(1-2): 121-7.

The authors of this publication reported that *Giardia lamblia* has the ability to modify several of its cellular proteins by isoprenylation. Protein isoprenylation and cell growth were inhibited in a dose dependent manner with complete inhibition obtained by concentrations of compactin ≥200 uM (~80 ug/ml) and mevinolin (data were shown for compactin only). This inhibition due to HMG-CoA reductase inhibitors was completely reversible by the addition of 2 mM mevalonate to the culture medium.

Lovastatin and Simvastatin Antimicrobial Activity Against Parasites:

Grellier, P., et al. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors lovastatin and simvastatin inhibit in vitro development of Plasmodium falciparum and Babesia divergens in human erythrocytes. Antimicrob Agents Chemother. 1994 May; 38(5): 1144-8.

In this publication, the authors evaluated the ability of lovastatin and simvastatin to inhibit, *in vitro*, growth and development of *Plasmodium falciparum* and *Babesia divergens*, the causative agents of human malaria and bovine babesiosis, respectively. *B. divergens*, in some cases, causes disease in humans.

Asynchronous parasite cultures (0.5% parasitemia and 1% hematocrit) of *P. falciparum* were maintained on human type O^+ RBC in RPMI 1640 culture medium supplemented with 27.5 mM $NaHCO_3$, 25 mM HEPES buffer (pH 7.4), 11 mM glucose, and 10% human O^+ serum in an atmosphere of 3% CO_2, 6% O_2, and 91% N_2 at 37° C. The *B. divergens* isolates were maintained *in vitro* in the same manner as *P. falciparum* except the cultures contained 1% parasitemia rather than 0.5%. Cultures were treated with lovastatin or simvastatin at various concentrations for 24 h. Parasite growth was estimated in lovastatin or simvastatin treated cultures either by [3H]hypoxanthine incorporation for 18 and 16 h, respectively, or by Giemsa-stained smears made at the end of the experiment. Results are shown in the following table.

Table I from Grellier, et al., 1994

Antiparasitic activities of HMG-CoA reductase inhibitors

	Mean IC_{50} ($ug ml^{-1}$) ± SD	
Parasite strain	Lovastatin	Simvastatin
P. falciparum		
F32/Tanzania	15.7 ± 6.5[a]	16.2 ± 3.6[a]
FcB.1/Columbia	13.6 ± 3.7[b]	12.8 ± 2.5[b]
B. divergens		
Rouen 1987	8.4 ± 0.3[b]	5.0 ± 0.4[b]
Weybridge 8843	ND[c]	5.8[d]

[a] From four experiments.
[b] From three experiments.
[c] ND, not determined.
[d] From two experiments.

Similar IC_{50} values were obtained for lovastatin and simvastatin against the plasmodium strains; both IC_{50} values were in the range of 10 to 20 ug/ml (~25 to 50 uM). The drugs were equally effective against the chloroquine-susceptible F32/Tanzania stain and the chloroquine-resistant FcB.1/Columbia strain. IC_{50} values for *B. divergens* isolates were in the range of 5 to 10 ug/ml (~12.5 to 25 uM) and suggest no difference in sensitivity between the two strains tested.

Subsequent inhibition assays with 6-h-pulse incubations of simvastatin with *P. falciparum* synchronized cultures showed that the trophozoite stage of the erythrocytic life cycle is the stage at which the parasite is most susceptible to simvastatin. Cytotoxic effects giving a complete inhibition of growth were observed for all parasite stages only with drug concentrations above 50 ug/ml (~125 uM). Reversal of parasite growth inhibition by excess of exogenous mevalonate was unsuccessful and may have been due to the inability of non-drug treated *P. falciparum* infected RBC to incorporate [14C]mevalonate. This observation suggests that the parasite is not capable of mevalonate uptake from the assay medium.

From these studies, the authors concluded that the achievable concentrations of HMG-CoA reductase inhibitors in human plasma are unsuitable for a blood eradication of malaria by the current usage of this cholesterol-lowering agent.

Simvastatin Antimicrobial Activity Against Parasites:

Coppens, I., et al. Activity, pharmacological inhibition and biological regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in Trypanosoma brucei. Mol Biochem Parasitol. 1995a Jan; 69(1): 29-40.

In this study, the authors measured, *in vitro*, the activity of HMG-CoA reductase in the bloodstream form and the culture-adapted procyclic form (insect form) of *Trypanosoma brucei*, the causative agent of sleeping sickness in humans. Synvinolin (simvastatin) was used as a tool to study the regulation of the activity of both HMG-CoA reductase and the abundance of low density lipoprotein (LDL) receptors exposed on the parasite cell surface. In the process, the effect of simvastatin on parasite growth and survival was determined. Simvastatin inhibited the growth of both procyclic and bloodstream forms. In lipoprotein free medium the exponential growth of the procyclics was reduced 2-fold and the sensitivity to synvinolin was enhanced approximately 20-50%. The effect was dose-dependent and increased with time of exposure to the inhibitor (Table 1).

Table 1
IC_{50} (uM) of synvinolin on the growth of *Trypanosoma brucei* and rat foetal fibroblasts in culture

	Bloodstream forms	Procyclic forms		Rat foetal fibroblasts
Lipoproteins in the medium	+	+	-	+
Exponential doubling time(h)	8-9	14	28	22
Incubation time(h)				
24	NT	55±7	39±9	160±25
48	26±4	50±6	27±8	75±9
69	NT	25±8	18±6	51±7

Trypanosomes were grown as described in Materials and Methods in medium containing 10% complete serum (+) or lipoprotein-free serum (-), in the presence of increasing concentrations of synvinolin. At the indicated times, the number of typanosomes was estimated in a haemocytometer, while protein content of adherent fibroblasts was measured by the Lowry assay. Values are means ± SD of IC_{50}, calculated from three separate experiments (NT, not tested).

In addition, growth of procyclics in complete serum showed similar IC_{50} values for 4 other inhibitors tested (compactin, mevinolin, fluvastatin and RG 12561; 53±10 at 40 h, 22±3 uM at 69 h of culture; combined means ±SD). However, growth inhibition due to simvastatin was reversible by products of the mevalonate pathway or by low-density lipoprotein as shown in Table 2 below.

Table 2
Reversal of procyclic growth inhibition due to synvinolin by products of the mevalonate pathway or by low-density lipoprotein

Medium	Growth (% of control)	
	Procyclics	Bloodstream forms
Control	100%	100%
Synvinolin	51±6%	46±3%
Synvinolin + mevalonate (20 mM)	99±7%	88±7%
Synvinolin + squalene (100 uM)	81±5%	41±13%
Synvinolin + cholesterol (100 um)	89±9%	45±11%
Synvinolin + LDL (300 nM)	97±6%	95±7%

Procyclics were first incubated at 28° C in 10% of lipoprotein-free serum, while bloodstream forms were incubated at 37° C in 10% of complete serum, both with or without 25 uM synvinolin, for 40 h. After synvinolin priming, the indicated products of the mevalonate pathway or LDL were added in the medium, and cells were further incubated for 48 h. Finally, the number of trypanosomes was estimated in a haemocytometer. Results are means ± SD of three experiments and expressed in % of control growth, where 100% corresponds to 5.5 10^{-6} ml^{-1} procyclics and 2.5 10^{-6} ml^{-1} bloodstream forms.

Interpretation of these data suggest that synvinolin inhibition of growth is reversed in procyclic forms by mevalonate, squalene, cholesterol and LDL whereas in bloodstream forms growth inhibition is reversed only by mevalonate and LDL.

Coppens, I., et al. Exogenous and endogenous sources of sterols in the culture-adapted procyclic trypomastigotes of Trypanosoma brucei. Mol Biochem Parasitol. 1995b Jul; 73(102): 179-88.

In this paper, the authors extend their work reported in their previous publication. They have demonstrated that procyclics can synthesize their sterols as well as use imported exogenous cholesterol by LDL endocytosis through specific receptors and incorporate this lipid into their membranes. Major changes in the culture medium, such as supplementation with excess LDL, total removal of lipoproteins, or exposure to simvastatin have the capacity to induce modifications in the rate of sterol biosynthesis and in the composition of membranes, as well as modify procyclics' growth rate. These data suggest that procyclics can adapt to extremely different media, so as to maintain a regulated supply of sterols

Miscellaneous Lovastatin Antimicrobial Activity Studies:

Numerous additional publications with limited information concerning lovastatin antimicrobial activity were identified in the published literature and are cited collectively immediately below this paragraph. The majority of these publications employed lovastatin as a molecular tool in molecular biology studies relative to the elucidation of isoprenoid and steroid biosynthesis mechanisms. Both individually and collectively, these data were not considered as relevant for the purpose of determining reclassification of lovastatin as an antibiotic drug. However, to complete the literature record, they are cited in this report in the event that subsequent discussion, relevant to the consideration of lovastatin's reclassification as an antibiotic, would benefit by their inclusion.

Bard, M., et al. Isolation and characterization of mevinolin resistant mutants of Saccharomyces cerevisiae. J Gen Microbiol. 1988 Apr; 134(Pt4) 1071-8

Koning, AJ., et al. Different subcellular localization of Saccharomyces cerevisiae HMG-CoA reductase isozymes at elevated levels corresponds to distinct endoplasmic reticulum membrane proliferations. Mol Biol Cell. 1996 May; 7(5): 769-89.

Lum, PY., et al. Molecular, functional and evolutionary characterization of the gene encoding HMG-CoA reductase in the fission yeast, Schizosaccharomyces pombe. Yeast. 1996 Sep 15; 12(11): 1107-24.

Ng, WL., et al. Minimal replication origin of the 200-kilobase Halobacterium plasmid pNRC100. J Bacteriol. 1993 Aug; 175(15): 4584-96.

Rostand, KS., et al. Cholesterol and cholesterol esters: host receptors for Pseudomonas aeruginosa adherence. J Biol Chem. 1993 Nov 15; 268(32): 24053-9.

Smith, SJ., et al. Transcriptional regulation by ergosterol in the yeast Saccharomyces cerevisiae. Mol Cell Biol. 1996 Oct; 16(10): 5427-32.

Taraboulos, A., et al. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform [published erratum appears in J Cell Biol 1995 Jul; 130(2): 501]. J Cell Biol. 1995 Apr; 129(1): 121-32.

Vanderplasschen, A., et al. The replication in vitro of the gamma herpesvirus bovine herpesvirus 4 is restricted by its DNA synthesis dependence on the S phase of the cell cycle. Virology. 1995 Nov 10; 213(2): 328-40.

DISCUSSION

Data useful for the analysis of whether a drug possesses antimicrobial activity sufficient to warrant its classification as an antibiotic drug product may be obtained from a variety of studies. These studies may include data generated from human clinical trials, animal models and/or from *in vitro* cell cultures. Obviously, data from adequate and well controlled human clinical trials, wherein the antibiotic properties of a drug product have been well characterized, would be the best source of information upon which to base a decision. In the absence of human clinical data, one has two choices with respect to drug classification decision making: 1) determine that the drug is a non-antibiotic drug because relevant human data are unavailable, or 2) utilize preclinical antimicrobial activity data extrapolated to relevant human use circumstances, where possible, in place of human data. Antimicrobial activity associated with lovastatin or related "statin" class of drugs from human clinical studies has not been reported in the literature. Therefore, option 2 has been addressed in this report, recognizing that management may determine a decision based upon option 1.

Ideally, one should have standardized and validated preclinical models for the determination of antimicrobial activity. The term, validation, refers to the circumstances where activity data developed from preclinical models are reproducible and have been shown to be predictive and to correlate with activity subsequently determined in human clinical trials. Unfortunately, the preclinical assays used for generation of antimicrobial data for HMG-CoA reductase inhibitors have been neither standardized nor validated. Consequently, considerable care should be taken when making attempts to determine relevance of preclinical activity data for human drug use parameters.

As a first step in the decision making process for classification of a drug as an antibiotic drug, a clear target definition of antibiotic drug should be determined. As discussed in the background section of this report, the legal definition of an antibiotic drug leaves some room for interpretation from at least two perspectives. First, the species of microorganisms that must be inhibited by a drug product have not been specified. Second, the term "... inhibits in dilute solution ..." does not include an interpretation as to the meaning of "dilute solution." It is recognized that there may be several alternative interpretations applied to this meaning. However, for the purpose of this data analysis and report, the term "... inhibits in dilute solution ..." is interpreted as the drug concentration in preclinical studies that elicits inhibitory activity against microorganisms that correlates with clinically relevant human tissue drug concentrations. Human tissue drug concentrations considered relevant are those that are achieved from doses administered to the human target populations for the indicated use of the drug. The data provided in the Pharmacology section of this report suggest that the target tissue drug concentration of relevance for lovastatin and simvastatin antimicrobial activity should be ~0.1 uM

Lovastatin and simvastatin *in vitro* antimicrobial activity was evaluated against a variety of bacteria, viruses, yeasts, fungi, and parasites as summarized in Tables 1-3. None of the microorganisms evaluated in these studies was inhibited by concentrations of lovastatin *in vitro* that were ≤ 0.1 uM, the target concentration as specified in the definition of dilute solution. However, several different species of microorganisms, including *H. holobium*, *H. volcanii*, HIV, *R. glutinis*, *S. salmonicolor*, *P. blakesleeanus*, *T. cruzi* amastigotes, and *S. mansoni*, were inhibited at 3- to 25-fold greater concentrations than the 0.1 uM target. The remaining microorganisms were inhibited only by lovastatin *in vitro* at concentrations more than 50-fold (range 50- to 1,900-fold) greater than that identified in the target definition of dilute solution. Simvastatin antimicrobial activity was evaluated only in parasites (Table 3), the growth inhibition of which required concentrations at least 125-fold (range 125- to 1,250-fold) greater than the target definition stated above. Reports of pravastatin antimicrobial activity were not found in the published literature.

The majority of the *In vitro* studies described in this report employed an experimental design that utilized minimal media, supplemented with either low concentrations of serum or lipoprotein depleted serum, for microorganism growth. This fact imposes a serious limitation upon interpretation of these data with respect to potential *in vivo* lovastatin antimicrobial activity. For example, *in vitro* growth inhibitory effects of the HMG-CoA reductase inhibitor, compactin, on Chinese hamster ovary cells (CHO) were shown to be dependent upon the amount of low density lipoprotein (LDL) and mevalonate present in the growth medium (Goldstein, et al., 1979; cited in a review by Brown and Goldstein, 1980). They reported that in the presence of either 2 uM or 40 uM compactin and in the absence of both LDL and mevalonate, CHO cells failed to grow. On the other hand, growth inhibition of cells treated with 2 uM compactin was reversed by the addition of 25 ug/ml LDL, but not by the addition of 0.5 mM mevalonate, to the culture medium. When cells were treated with 40 uM compactin, neither 25 ug/ml LDL alone nor 0.5 mM mevalonate supported growth. However, the combination of 25 ug/ml LDL and 0.5 mM mevalonate restored full growth of CHO cells even in the presence of 40 uM compactin. Interpretation of these data shows that the MIC of compactin can be increased by a minimum of 20-fold (i.e., 2 uM to 40 uM), and perhaps more, depending upon the composition of the growth medium with respect to LDL and mevalonate content. The concentrations of LDL and mevalonate necessary to reverse HMG-CoA reductase inhibition of cell growth are variable. In the absence of cholesterol, cell growth requires large amounts of mevalonate, most of which is channeled into cholesterol biosynthesis. When cholesterol is present in saturating amounts, only a small amount of mevalonate, required for isoprenoid biosynthesis, is necessary to support cell growth. In many of the publications reviewed for this report, the antimicrobial activity of lovastatin was shown to be reversed by the addition of varying amounts of mevalonate or other products of the steroid or isoprenoid biosynthetic pathways, such as LDL, cholesterol, farnesol, and squalene. Moreover, reversal of growth inhibition required less mevalonate in studies that employed higher level of serum in their growth medium. Interpretations from these data suggest that antimicrobial activity of HMG-CoA reductase inhibitors determined from these *in vitro* study results would be substantially diminished if the assay media employed in these studies were not limited in serum and contained concentrations of mevalonate and LDL normally present *in vivo*. These observations would suggest that HMG-CoA reductase inhibitors should exhibit significantly less antimicrobial activity *in vivo* than that observed in these *in vitro* studies.

Only four murine animal model *in vivo* studies containing lovastatin antimicrobial activity data were identified in the literature; two studies on *S. mansoni* and two on *T. cruzi*. In *S. mansoni*, adult schistosome survival was reported to be reduced 96-100% in mice fed 0.2% lovastatin (640 mg/kg) in their diet for 14 days (Chen, et al., 1990). At 250 mg/kg, adult schistosome survival was reported to be unaffected although egg production was inhibited 45.4% (Vandewaa, et al., 1989). In mice treated with 100 mg/kg lovastatin, egg production was unaffected while at 50 mg/kg egg production was enhanced. Thus, it appears that considerably high levels of lovastatin are required to inhibit *S. mansoni* adult schistosomes and egg production *in vivo*, in spite of the *in vitro* sensitivity of *S. mansoni* also reported in these studies.

Conflicting data were reported concerning lovastatin's antimicrobial activity against *T. cruzi* infection in mice. Urbina, et al., 1993, reported that lovastatin, as a single drug, was incapable of inhibiting parasitemia in infected mice, although a dose of 20 mg/kg/day administered for 7 days increased survival 50% over untreated, infected, control mice. In contrast, Brener, et al., 1993, reported that lovastatin at 100 mg/kg/day exacerbated parasitemia approximately 3-fold

over untreated controls and failed to provide a survival benefit These data are in contrast to results obtained from *in vitro* studies where, at least for *T. cruzi* amastigotes, growth inhibitory concentrations were only 10-fold greater than the target definition of "... inhibits in dilute solution . .." Lovastatin, at 1 uM, was reported to eliminate *T. cruzi* amastigotes from *in vitro* cell cultures within 192 h; whereas, epimastigotes required concentrations of 25 to 125 uM (Urbina, et al., 1993; Florin-Christensen, et al., 1990). These results are consistent with the prediction that *in vivo* antimicrobial activity of HMG-CoA reductase inhibitors would be substantially less than that reported from the *in vitro* studies.

Several publications also contained data on the antimicrobial effect of lovastatin in combination with azoles. Azoles are known to inhibit ergosterol biosynthesis in yeasts and fungi. The rationale for the study design was two-fold. First, lovastatin had been reported to inhibit sterol biosynthesis in the yeast, *S. cerevisiae*, and in the process increase cell membrane permeability to exogenous sterols. It was anticipated that this increase in cell permeability would extend to azoles present in the cultures, thus, potentiating antimicrobial activity of the azole. Secondly, lovastatin and azoles inhibit two separate enzymes involved in the biosynthetic pathway of ergosterol, a sterol required for growth of fungi, yeasts and some parasites. Exposure of a microorganism to drugs capable of inhibiting two separate targets in ergosterol biosynthesis was anticipated to be able to maintain antimicrobial activity of the azole while permitting lower, perhaps non-toxic, doses of azoles to be used in the treatment of infections.

In general, results from *in vitro* studies showed that lovastatin in combination with azole drugs resulted in a synergistic antimicrobial interaction against several microorganisms. However, the same cautions pertaining to the *in vitro* lovastatin antimicrobial data referred to above should be applied to these combination studies. In addition, combination drug activity observed in a murine model of parasite infection was less impressive with respect to antiparasitic effects. One report suggested a slight reduction in ketoconazole required to eliminate *T. cruzi* parasitemia in mice when used in combination with lovastatin (Urbina, et al., 1993). However, a separate report suggested an antagonistic interaction for lovastatin and ketoconazole when used in combination against the same species of microorganism (Brener, et al., 1993).

Unfortunately, the *in vivo* results reported in the above studies are further complicated by the fact that the authors failed to take into consideration a very major concern. Ketoconazole and itraconazole are known to inhibit the cytochrome P450 3A enzyme family responsible for the metabolism of lovastatin (Wang, et al., 1991; Back, et al., 1992; Rotstein, et al., 1992). Inhibition of this enzyme by itraconazole has been shown to increase the concentration of lovastatin by 20- to 30-fold in normal human subjects administered 200 mg itraconazole daily for 4 days followed by a single 40 mg dose of lovastatin on day four (Neuvonen, et al., 1996). In one of the 12 subjects in the study, creatine phosphokinase increased 10-fold within 24 hours following administration of the lovastatin dose, indicating skeletal muscle toxicity. This increase did not occur when the subject was given the same lovastatin dose four weeks later without itraconazole. Moreover, in transplant patients taking lovastatin and cyclosporine, a drug that inhibits cytochrome P450 enzyme CYP 3A4, serious myopathies (attributed to increased plasma lovastatin concentrations) have been reported that can be controlled by lovastatin dose reduction and careful monitoring of lovastatin plasma levels (Arnadottir, et al., 1993).

While the authors of the studies for the evaluation of antimicrobial activity associated with lovastatin in combination with ketoconazole focused on the potential to reduce interference with hepatic function and testosterone production associated with high doses of ketoconazole, they failed to consider the effects of ketoconazole on increasing tissue lovastatin concentrations and the potential for lovastatin induced toxicity exacerbation. It is not clear from the data available if lovastatin concentrations, when reduced sufficiently to avoid potential toxicity reactions, would elicit a synergistic response with respect to ketoconazole's antiparasitic activity to be meaningful. Moreover, the concept of lovastatin's ability to potentiate the activity of another drug that is not an antibiotic may be irrelevant to the discussion.

Table 1. Summary of Lovastatin *in vitro* Activity Against Bacteria and Viruses.

Microorganism Inhibited	Inhibitory Concn. (uM)	Reference	Comments
Escherichia coli	>68.3	Zhou, et al., 1991	Inhibition of growth was not achieved. Eubacteria do not utilize acetyl CoA and mevalonate in biosynthesis of isoprenoids.
Halobacterium holobium	1-2	Cabrera, et al., 1986	Cells of the genus, *Halobacterium*, require >15% NaCl for growth. Inhibition reversed by 4 mM mevalonate.
Halobacterium volcanii	1-2 20-40	Lam, et., 1989	In minimal medium, MIC is 1-2 uM. In enriched medium, MIC is 20-40 uM.
Murine Leukemia Virus (MuLV)	ND*	Overmeyer, 1992	2.5 uM lovastatin prevented maturation of MuLV's glycoprotein precursor, $gPr90^{env}$, to the mature envelope glycoprotein, $gp70^{env}$. Inhibition of virus infectivity was not reported.
Human Immunodeficiency Virus (HIV)	0.3	Maziere, et al., 1994	H9 cells were adapted to grow in medium supplemented with 1% serum to limit exogenous cholesterol. Virus inhibition was determined by a reverse transcriptase assay. Reverse transcriptase was reduced ~10-fold after lovastatin treatment compared to untreated, infected controls.
Measles Virus	ND	Malvoisin, et al., 1990	Measles virus induced syncytia in Vero cells was inhibited at ~15 uM lovastatin. Inhibition of measles virus infectivity was not reported.

*Not determined.

Table 2. Summary of Lovastatin *in vitro* Activity Against Yeasts and Fungi

Microorganism Inhibited	Inhibitory Concn. (uM)	Reference	Comments
Rhodotorula glutinis	~0.25	Ikeura, et al., 1988	Cells were grown in 0.67% yeast nitrogen base with 0.5% glucose. Cell inhibition with compactin was shown to be reversed, except for *C. matritensis*, by addition of 10 mM mevalonate to the culture medium.
Sporobolomyces salmonicolor	~2.5		
Aessosporon salmonicolor	~5.0[a]		
Citeromyces matritensis	~5.0[a]		
Saccharomyces cerevisiae	≥190	Lorenz, et al, 1990	Lovastatin at ~25 uM, in combination with ketoconazole, clotrimazole or miconazole, decreased the MICs of these azoles 6-, 10- and 32-fold, respectively, suggesting a synergistic antimicrobial activity between lovastatin and azoles against *S. cerevisiae*.
Candida albicans VA	~125	Sud, et al, 1985	Lovastatin, at concentrations between ~2 and 62.5 uM, gave a fourfold or greater reduction in ketoconazole MICs when used in combination studies. However, a fourfold reduction for *C. tropicalis* and *T. glabrata* was not obtained. These data were generated utilizing completely synthetic media.
Candida albicans 7.22	~250		
Candida tropicalis	>250		
Torulopsis glabrata	>250		
Aspergillus fumigatus 173	~15		
Aspergillus fumigatus	~15		
Aspergillus niger	~30		
Rhizopus rhizopodiformis	~125		
Phycomyces blakesleeanus	1	Bejarano, et al., 1992	Fungus was grown on minimal agar medium. The observed inhibition by lovastatin was reversed by the presence of 10 mM but not 1 mM mevalonate.
Physarum polycephalum	≥25	Engstrom, et al., 1989	Inhibition of protein synthesis, DNA synthesis, nuclear division and plasmodia growth could be partially reversed by the addition of mevalonate at concentrations ≥ 0.4 mM.

[a]Inhibition determined with compactin only (lovastatin is an analogue of compactin)

Table 3. Summary of Lovastatin and Simvastatin *in vitro* Activity Against Parasites

Microorganism Inhibited	Inhibitory Concn. (uM)	Reference	Comments
Trypanosoma cruzi	25 to 125	Florin-Christensen, et al., 1990	Lovastatin inhibited growth in a dose dependent fashion; at 25 and 75 uM, growth was progressively inhibited. However, 125 uM was required to kill most of the trypanosomes. Squalene (100 uM), but not cholesterol, was able to reverse growth inhibition produced by 25 to 75 uM lovastatin.
Trypanosoma cruzi			
epimastigotes	50 to 75	Urbina, et al., 1993	Growth was reduced 20-30% at lovastatin concentrations of 7.5 uM. Complete inhibition was observed at 50 and 75 uM after 144 h and 96 h of culture incubation, respectively. Trypanocidal concentration of lovastatin was reduced by a factor of 10 when incubated in combination with 0.1 uM ketoconazole.
amastigotes	>1		Concentrations of lovastatin greater than 1 uM were cidal for the Vero cells used to maintain the amastigotes in culture. Only 30% growth inhibition was observed for the amastigote at 1 uM lovastatin concentration. Lovastatin 0.75 uM in combination with 1 nM ketoconazole eliminated amastigotes from cell cultures after 192 h of incubation. Terbinifine 1 uM, required 25 uM lovastatin for complete growth inhibition.
Leishmania donovani			
promastigotes	~200	Haughan, et al., 1992	*L. amazonensis* was the strain most sensitive to lovastatin. In combination with miconazole, the IC_{50} values of each drug could be reduced 2- to 10-fold compared to when used as a single agents. At 25 uM, lovastatin had little effect on amastigotes in macrophage culture. Due to drug solubility problems, higher concentrations could not be tested.
Leishmania amazonensis			
promastigotes	~50		
amastigotes	>25		
Schistosoma mansoni	>100	Morrison, et al., 1986	Egg production in *S. mansoni* grown *in vitro* with 50% horse serum could be depressed ~50% at 1 uM lovastatin. However, at 100 uM complete inhibition of egg production was not obtained and growth inhibition of adult mating pairs was minimal. Egg production inhibition was not reversed by coincubation with 100 uM cholesterol. Reversal by mevalonate was not evaluated.
Schistosoma mansoni	10	Vandewaa, et al., 1989	Egg production in *S. mansoni* grown *in vitro* with 50% horse serum was inhibited ~5-fold at 10 uM lovastatin. Inhibition by lovastatin could be reversed by the addition of either farnesol or mevalonate at a concentration of 80 uM.
Schistosoma mansoni	1 to 10	Chen, et al., 1990	Lovastatin inhibition of adult schistosome motility and lactate production was time and dose dependent. At 3 days incubation, 10 uM lovastatin reduced motility and lactate production >50%; at 11 days of culture, doses of 1-10 uM inhibited activity nearly 90%. Inhibition was reversed in the presence of 50 uM mevalonate.
Giardia lamblia	≥200	Lujan, et al., 1995	Protein isoprenylation and cell growth were inhibited in a dose dependent manner with complete inhibition obtained by concentrations of compactin and mevinolin ≥200 uM. Data for mevinolin were not shown. The inhibition could be reversed by the addition of 2 mM mevalonate to the culture medium.
Plasmodium falciparum			
F32/Tanzania	>125[b,c]	Grellier, et al., 1994	IC_{50} values for *P. falciparum* were in the range of 25 to 50 uM. However, complete inhibition of growth was observed only with concentrations >125 uM. Simvastatin was tested against these organisms with similar results obtained.
FcB/Columbia	>125[b,c]		
Babesia divergens			
Rouen 1987	~12.5 to 25[c]	Grellier, et al., 1994	Activity of lovastatin was not determined against the Weybridge strain.
Weybridge 8843	~15[b]		
Trypanosoma brucei brucei	>20	Andersson, et al, 1996	Lovastatin, at a concentration of 0.1 uM, blocked interferon gamma induced proliferative responses of *T. brucei brucei*. However, growth of non-stimulated parasites was not affected at concentrations as high as 20 uM.
Trypanosoma brucei			
bloodstream forms	25[b]	Coppens, et al., 1995a	Simvastatin inhibited the growth of both bloodstream and procyclic forms of *T. brucei* with IC_{50} values in the range of 25 uM and 18 to 55 uM, respectively. In lipoprotein free medium the exponential growth of the procyclics was reduced 2-fold and the sensitivity to simvastatin was enhanced approximately 20-50%.
procyclic forms	18 to 55[b] 22 to 53[c]		

[a]Not determined.
[b]Reported for simvastatin.
[c]Reported for lovastatin

Table 4 Summary of Lovastatin *in vivo* Activity Against Parasites

Microorganism Inhibited	Inhibitory Concn. (mg/kg)	Reference	Comments
Schistosoma mansoni	>250	Vandewaa, et al., 1989	Adult schistosomes were unaffected in mice treated with 250 mg/kg lovastatin orally for 10 days. Egg production was inhibited 45.4% in these mice. However, in mice treated with 50 mg/kg, egg production was enhanced (degree of enhancement not reported) over that observed in control mice.
Schistosoma mansoni	640	Chen, et al., 1990	Adult schistosome survival was reduced 96-100% in mice fed a diet consisting of 0.2% lovastatin (640 mg/kg/day) for 14 days.
Trypanosoma cruzi	ND*	Urbina, et al., 1993	Lovastatin as a single drug was incapable of inhibiting parasitemia in infected mice. However, at a dose of 20 mg/kg/day administered for 7 days, mouse survival was promoted 50% over untreated controls. When a low dose of lovastatin and ketoconazole were combined, 100% survival and almost complete suppression of parasitemia were reported.
Trypanosoma cruzi	ND	Brener, et al., 1993	At a dose of 100 mg/kg/day administered for 19 days post-infection, lovastatin exacerbated parasitemia approximately 3-fold over untreated controls and failed to provide a survival benefit associated with treatment. In combination studies with ketoconazole, lovastatin appeared to elicit an antagonistic response with respect to ketoconazole's antiparasitic activity.

*Not determined.

CONCLUSIONS

Lovastatin, simvastatin, and pravastatin are the only anti-hypercholesteolemia drug products in CDER that meet the part of the antibiotic drug definition "... produced by microorganisms or any chemically synthesized equivalent ...".

Antimicrobial activity associated with lovastatin, simvastatin, and pravastatin in humans studies was not found in the published literature.

Antimicrobial activity associated with pravastatin was not found in the published literature.

Antimicrobial activity associated with lovastatin and simvastatin from *in vitro* and *in vivo* studies was reported.

The concentration of lovastatin and simvastatin in plasma obtained from human subjects administered the maximum approved dose daily for 17 days, the target parameters relevant for the antibiotic drug definition "... inhibits in dilute solution ...", was estimated to be ~0.1 uM.

None of the bacteria, viruses, yeasts, fungi, or parasites evaluated in the *in vitro* studies conducted for the assessment of antimicrobial activity was inhibited by lovastatin or simvastatin concentrations of 0.1uM.

Several species of microorganisms were inhibited at concentrations of lovastatin 3- to 25-fold greater than the target lovastatin tissue concentration of 0.1 uM. The remainder were reported to be inhibited at concentrations of 50- to 1,900-fold greater than 0.1uM.

The majority of the *in vitro* studies utilized assays that severely restricted serum and lipoprotein. Growth inhibition by HMG-CoA reductase inhibitors is known to be significantly enhanced when assayed in limited serum or lipoprotein conditions.

Growth inhibition can be reversed by the addition of LDL and mevalonate to cultures.

These facts suggest that the *in vitro* assays used in these studies are artificial systems and that the antimicrobial activity observed for lovastatin and simvastatin in these assays would be substantially diminished in an *in vivo* environment.

As predicted, lovastatin antimicrobial activity in a murine model of *Schistosoma mansoni* and *Trypanosoma cruzi* infections was reported to be minimal.

If the target human tissue lovastatin and simvastatin concentration of 0.1 uM is used as a basis for the definition of "... inhibits in dilute solution ...", the available data are insufficient to support the conclusion that lovastatin, simvastatin, and pravastatin have sufficient antimicrobial activity to warrant their reclassification as antibiotic drugs.

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